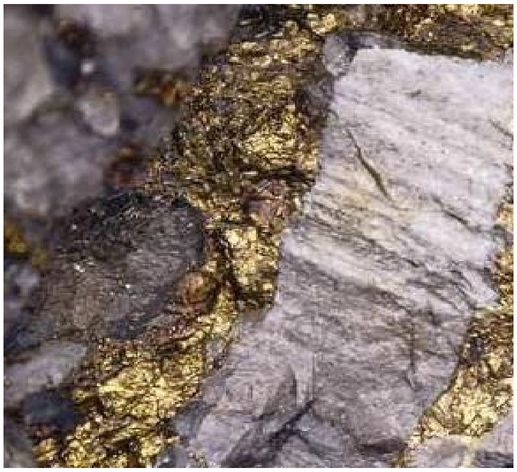

iGEM TORONTO Policy and Practices 2016

Miners Against Malaria:

An Efficient Paper- Based Biosensor Approach to the Early Diagnosis and Prevention of Malaria Amongst Artisanal and Small-Scale Gold Miners

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| Introduction

A white paper is a working, briefing document that aims to broadly inform the reader about a problem along with its scope and provides a solution. iGEM Toronto Policy and Practices (P&P) team decided to opt for a white paper as it allowed us to think about our subject broadly in hopes that we can narrow our focus when forming our solution. We also chose the white paper because it is meant to be an authoritative report rather than a proposal, which can provide a skewed outlook in terms of persuasion. After developing the wet and dry lab projects, iGEM Toronto aimed to design a project that would combine the two in a novel way for a well-designed application. As a working document, we used the white paper as a means of managing all of our research, keeping track of our sources and to continually revisit our ideas as an iterative process when we came across helpful insights.

Human Policy & Practices focuses on the subject of "how your work affects the world, and how the world affects your work" (Peter Carr, Director of Judging, iGEM). After brainstorming potential impacts of the cell-free paper-based biosensor coupled with a smartphone app, iGEM Toronto P&P divided into groups based on specific research interests: Ethics, Geology, Business, Biosensor Design, and Malaria.

During our "desk research" phase, we allocated members to look into these research interests and focused on questions relating to the value of gold, the gold supply chain, and the mining industry. Another issue that we were having issues with had to do with how to use the biosensor in the field. Our interest in artisanal and small-scale gold mining (ASGM) peaked when we discovered a correlation between ASGM and malaria prevalence in endemic regions such as Brazil (de Andarde et al., 1995; Duarte & Fontes, 2002; de Oliveira et al., 2013), Suriname (Antonius-Smits et al., 1999), Ghana (Asante et al., 2011), Peru (Asante et al., 2011), the Ivory Coast (Knobl-auch et al., 2014) and Kenya (Imbahale et al, 2011). Since artisanal mining supplies 15-20% of global minerals and metals, we were interested in finding a way for our genetically-engineered technology to address this issue and impact a part of the industry that may be unaccounted for. This document describes our ongoing research process.

1 | Ethics

1.1 Dichotomy between livelihood and well-being

For communities around the world, artisanal and small-scale mining (ASM) is an important source of livelihood (Hinton, 2003). At a small scale, this practice may inflict costs not only on the environment, but on human health as well. Mercury or cyanide is often used by artisanal miners to separate gold from the ore (Hinton, 2003). Mercury is amalgamated to gold in a process that releases 25-30% of the mercury into the environment (Veiga, 2009). As a known neurotoxin, mercury is also biopersistent, remaining in the environment for a long time (Ullrich, 2010). The repercussions of mercury affect the miners directly, but can also reach the surrounding communities indirectly via the food chain (Hinton, 2003). Cyanide, the alternative to mercury, comes with its own set of consequences. It is an industrial chemical and a rapid-acting poison that must be carefully handled (RTI International, 2006).

The risk of harmful chemicals combined with the evident physical occupational hazard associated with mining places workers of the ASM industry at a stalemate. Artisanal miners are presented with a choice between their livelihoods or their well-being. When one's source of income is dependent on this choice, one's perception of safety, defined as "the risk that is known and judged as acceptable," may be skewed (Naagarazan, 2006). The risks associated with gold mining are evident but workers continue to put themselves in unsafe situations. This may be explained by the theory of cognitive dissonance, the stress experienced when one's behaviour contradicts one's thoughts and beliefs. As a way of coming to terms with the decisions one makes, risks may be distorted and subsequently perceived as less harmful. Reduced apparent risk removes the incentive to disengage in these unhealthy practices, leading miners to compromise their well-being for their livelihood.

1.2 Society and Conflicts

Gold is one of the few commodities that is extremely valuable across cultures. Almost all countries in the world store a good deal of their wealth in the form of a gold reserve. Additionally, the use of gold in mythology, such as the golden fleece, and in the artifacts of several cultures, is a testament to its value. This value stems from gold's rarity and various chemical properties such as its lustre. The majority of the gold on Earth is believed to have sunk to the core of the planet during its initial formation. As a result, gold is a relatively rare metal. Many people desire gold specifically because it can not possessed by many others due to its rarity. For some commodities, such as gold, people tend to draw joy by possessing something

that others find desirable but don't have. Thus gold's rarity is responsible in part for its high value. Moreover, gold is very lustrous which tends to draw the attention of humans and creates a strong desire to possess it. The luster of gold is largely responsible for society's obsession with gold.

Gold is among the array of minerals responsible for conflict in the Democratic Republic of Congo. The abundance of natural resources in Congo is a blessing voided by the history of wars fuelled by the scramble for minerals. Today, gold and other precious minerals are key sources of wealth for armed groups, with gold alone providing $44 to $88 million annually (Raj, 2011). Local Congolese are forced to mine in dangerous conditions by militias who maintain power through extortion, bribery, violence, and rape (Raj, 2011). These conflict minerals are often made into goods like cell phones and computers, and are sold to oblivious American consumers (Eichstaedt, 2011).

1.3 Ethical Impact of a Cell-Free Paper-based Biosensor

The proposed paper-based biosensor would increase the safety and efficiency of current prospecting techniques at the expense of promoting further mining. The benefit of the biosensor is three-fold. Firstly, the biosensor simplifies the prospecting procedure, allowing shorter prospecting time and minimizing exposure to unsafe conditions at this stage. Secondly, the biosensor allows for a more efficient identification of gold veins, which is conducive to the livelihoods of artisanal miners. Thirdly, the biosensor contains inactive genetic material that is easy and cheap to transport. On the other hand, simplifying the prospecting procedure inevitably facilitates gold mining. This may promote detrimental practices associated with mining, including environmental destruction, hazardous mining work, and conflict mineral mining. The question of who has rights to creating and distributing the biosensor needs to be asked to determine how it will be used broadly.

2| Geology

2.1 Prospecting Methods

Prospecting refers to the process of searching for mineral deposits. There are several ways this can be done:

I. Looking for surface formations, taking soil samples: Field prospectors go out and look for ore-forming systems based on visual aspects such as potassic or argillic alterations. Both of these are examples of metasomatism (chemical alterations of rock formations by hydrothermal or other fluids), or simply lustrous mineralization in outcrops. Metasomatism is often indicative of hydrothermal veins in which precious formations can occur. Potassic alterations often leave distinct salmon pink colourations and produce minerals such as micas, sericite, and orthoclase. These alterations are often associated with lode gold deposits. (Zharikov, 2007).

II. Seismic methods: There are 2 seismic methods that are used in gold prospecting: reflection seismology and seismic refraction. Reflection seismology involves the use of a surface acoustic source (eg. explosives) to map rock properties such as permeability, porosity, and to identify the presence of gas and fluids. The acoustic source sends propagating sound waves in all directions and some of this energy is reflected from subsurface materials. This energy is recorded by a geophone, a device that converts ground movement to voltage. Times and amplitudes of the voltage are used to interpret the subsurface (Candela). Seismic refraction, similar to seismic reflection in that it uses an acoustic source to generate energy. The difference between these two techniques is that seismic refraction uses equipment to measure the change in direction of the energy as it travels between media. Times of arrival are recorded as the energy is refracted along a second layer boundary, before it returns to the surface where more detectors are placed, and the new angle and time are recorded. This data results once again in mapping of the subsurface layers (Engineering Manual).

III. Physical Geodesy/Gravity: This involves analysis of gravity and the geopotential of the earth and how they relate to the shape of the Earth. A gravimeter utilizes a fine-tuned spring attached to a pendulum to measure the difference in gravity from a gravity reference. A gravity high or low indicates that there is an anomaly located in the subsurface that is denser than the surrounding country rock (e.g. sulphide deposit) or less dense (e.g. aquifer). This can be used to create 2D/3D images and density/material estimates. Airborne surveys are also feasible however they will sacrifice some precision for regional coverage and time.

IV. Magnetics: This method takes advantage of the magnetic field that an object in the subsurface has as a result of the earth's magnetic field upon its formation. One magnetometer is placed in a fixed location to take baseline measurements to correct for any variations caused by a change in inclination/declination, and solar interference among other variables. Simultaneously another magnetometer takes measurements in a grid of interest. Presence of a magnetic anomaly could indicate mineralization. This method is commonly used to image extrusive/intrusive igneous bodies and precipitated mineral formations. 2D/3D models are common goals of magnetic surveys.

V. Resistivity/Induced Polarization: Resistivity, or Electrical Resistance Tomography (ERT), is an imaging tool that uses electrodes and direct currents to determine subsurface electrical resistivity distribution. Electrodes are placed either on the ground or in boreholes in pairs; an electrical current is injected into the ground between one pair of electrodes, and as the current travels, voltage is recorded between another pair of electrodes. This is done in sequences through sets of electrodes in order to gather enough data for imaging. Sections of lower resistivity are possible indicators of metallic oxides or sulphide minerals. (Daily, 2004). Similar to ERT, Induced Polarization (IP) also injects electric currents into the ground between electrodes, but IP switches the current off part way through. A potential field is induced by the current flow, and is measured in both the on- and off-time, as a measure of chargeability, as transient voltage decay differs between materials. (GPX Surveys, 2016)

VI. Magnetotellurics (MT): MT is a geophysical method that measures the magnetic and electric fields around the earth. MT frequencies are a result of solar winds (low frequencies) and thunderstorms (high frequencies) interacting with the earth's magnetic field. A non-intrusive method, this type of survey excels at identifying subsurface structures, especially ore bodies and other conductive materials. This method also allows for the detection of resistivity anomalies. While 3D mapping is available, it requires immense computational power and thus 2D maps are more commonly produced amongst junior exploration companies.

VII. Ground Penetrating Radar (GPR): GPR transmits a high frequency pulse and measures the energy of the wavelet that bounces from the subsurface. Energy is only reflected (just like in seismic methods) through a change in medium (subsurface boundary). Two basic concepts of this method involve frequency and conductivity. Attenuation (depth penetration) will decrease as a higher frequency antenna is used or as conductivity goes up. Increasing frequency will also increase resolution. Running a GPR line results in what is called a slice (2D image). Multiple slices in different directions can be used to

create 3D models. This method can be very useful if the target anomaly has different properties than the surrounding material. (UBC)

VIII. Transient Electromagnetics (TEM)/Time-Domain Electromagnetics (TDEM): This method is used to measure the resistivity at depths of several hundred metres. TEM focuses on picking up the secondary electric fields resulting from an induced primary magnetic field interacting with the ground (conductor). When the primary field is shut off, the change in secondary field is measured. This method can be used to locate minerals and characterize them. TEM occurs through several steps. Initially a current is ejected into the Tx-loop resulting in a static primary magnetic field (Sorensen *et al.* 2006). Subsequently the current is turned off and the resulting change in the primary magnetic field creates an electromotive force in the surroundings. This electrical field creates a current in the ground resulting in a secondary magnetic field (Sorensen *et al.* 2006). Immediately after the transmitter is switched off this secondary magnetic field will be equivalent to the primary magnetic field which is now absent (Sorensen *et al.* 2006). Over time the resistance in the ground will weaken the current. Additionally the current density maximum moves progressively outwards and downwards further weakening the current (Sorensen *et al.* 2006). Following this, measured current signals just below the surface can reflect the resistivity of the top layers. Additionally as current diffuses deeper into the ground, readings give information about the resistivity of deeper layers (Sorensen *et al.* 2006).

IX. Borehole Geophysics (Well Logs): Well Logs essentially record formations, depths, and events while drilling a hole. Various instruments are lowered into the well while drilling occurs in order to record various variables versus depth. This can vary from radioactivity to porosity, and even resistivity. As such, Well Logging is a very versatile tool that can be used for many types of geological exploration. (Rigzone, 2016)

X. Geochemical Methods (Soil Sampling/Water Sampling): Much simpler than any geophysical methods, geochemical methods rely on taking direct samples from soils near potential deposits/in areas with proven success record for geochemical methods or streams with similar potentials. These samples are then brought to labs and are subject to various assay techniques (eg. fire assays, bottle roll cyanide leach for gold, four acid digest and AAS for gold, or aqua regia digest and AAS for gold). (ALS Minerals).

It is important to realize that none of these methods will give a definitive answer as to whether or not an ore is present however they do increase the likelihood that an ore will be found once a survey is conducted. Often several of these methods are combined in order to increase the certainty that there is an deposit present.

Methods used by artisanal miners are far less advanced, as the equipment required for many/all of the above methods, used primarily by junior and senior mining companies, can be extremely costly. Equipment is also often physically very large, requiring expensive and careful transport, and usually require extensive training for proper use. ("Mine & Mill Equipment Costs", 2016)

2.2 Gold Occurrence

Gold occurrence refers to the areas where gold is typically found. There are both primary deposits and secondary deposits. Primary deposits form when gold precipitates during chemical reactions between hydrothermal mineralizing solutions and rocks in the Earth's crust (Zhu *et al.*, 2011).

The 3 primary deposits are:

I. Primary hydrothermal veins: Formation of these deposits occurs upon interaction of wall rocks and hydrothermal fluid. Formation is dependent on temperature, pressure, pH, and fugacity of H_2S in the hydrothermal system (Frimmel, 2008). Gold tends to be concentrated in the vapor phase of fluids in high temperature and pressure conditions (Zhu *et al.*, 2011). These deposits are characterized by well-developed metal zonation pattern with a typical sequence of Fe to Fe-Cu to Cu-Pb-Zn to Pb-Zn-Ba-Au in an upwards and lateral sense (Frimmel, 2008). Concentration of these various metals depend on deposit location, temperature, pressure and pH. This pattern is a reflection of the solubilities of these metals at progressively lower temperatures (Frimmel, 2008).

II. Magma intrusion/intrusion deposits: Intrusion deposits are typically found in granites and igneous rock which often also have copper present in them. These minerals were either carried in liquid form with the magma or were part of the magma and forced into the rock walls. Magma degassing can release enough ore forming elements to form economic deposits of gold. The concentration of gold is 100-1000 times higher than that found in hydrothermal veins (Ulrich *et al.,* 1999; Pudack *et al.,* 2009; Seo *et al*., 2009).

III. Volcanic-exhalative sulphide deposits: This refers to a type of metal-sulfide ore deposit which are associated with and created volcanic-associated hydrothermal events in submarine environments. These deposits are formed on the seafloor around undersea volcanoes (Ulrich *et al*., 1999).

Additionally there is 1 type of secondary gold deposit: alluvial and eluvial deposits. This occurs when rocks containing the gold veins have been exposed and are eroding away. The gold

in these rocks is washed into creeks to form alluvial gold deposits. The gold is then further concentrated due to erosion by water. Since gold is heavier than most of the material moved in the river it can become concentrated and trapped in the river bed (Butt, 1997).

2.3 Fire Assays

Fire assays are commonly used to evaluate gold deposits. However now there exists several high-tech alternatives to fire assays such as INAA (instrumental neutron activation), GFAA (graphite furnace-atomic absorption), and *aqua regia* digestion (Hoffman *et al.*, 1998). The costs of these techniques vary, they range from $5-7 US for a 30 g sample to $35 for a 30 g sample (Hoffman *et al.*, 1998). Fire assays involve mixing a powdered sample with soda ash, borax, litharge, silica and potassium nitrate. Ag or Pd is then added and the mixture is heated to 1000-1200˚C. As the Pb and Ag (or Pd) melt they cause gold to accumulate at the bottom. Once poured into a mould and cooled the slag separates from the lead button. The gold is separated from Ag by dissolving it in nitric acid and the resulting mass is weighed (Hoffman *et al.*, 1998).

Fire assays are so frequently used because they yield high quality and reproducible results (Hoffman *et al.*, 1998). In addition fire assays are easily adaptable to a mine setting, they are relatively cheap and results are obtained quickly (Hoffman *et al.*, 1998). Unfortunately, sample mix ups are occasionally an issue with fire assays. Additionally the need to use blanks in fire assays decreases the reliability of results using small samples (Hoffman *et al.*, 1998).

In INAA samples are irradiated with neutrons causing any Au-197 that is present to become Au-198. When Au-198 decays back to Au-197 gamma radiation with an energy level specific to gold is emitted. This radiation is quantified using an analyzer to evaluate the gold sample (Hoffman *et al.*, 1998). Some advantages of INAA are that: it is simple and reliable since no chemical reaction is required, this method is nondestructive, and it does not require a blank to subtract making it accurate for small sample sizes (Hoffman *et al.*, 1998). INAA is also cost effective. The main disadvantage of this technique is the 7 day wait for sodium decay prior to analyses (Hoffman *et al.*, 1998).

The *aqua regia* dissolution procedure involves dissolution in aqua regia followed by a solvent extraction (Hoffman *et al.*, 1998). This technique is the cheapest gold-analytical technique and can be performed very quickly (Hoffman *et al.*, 1998).

3| Business

3.1 Gold Value Chain/Input-Output Structure

The input-output structure describes the process of going from raw materials to a finished product including interactions between firms involved in this process (DaSilva, 2013). In the gold mining industry this process starts with research. Mining companies must perform geophysical research such as gold prospecting, resource evaluation and reserve definition to determine if a commercially viable amount of gold exists at that site (DaSilva, 2013). These companies must also perform market research which involves using commodity prices and demand to determine if the resources discovered will be worth mining (DaSilva, 2013). Subsequently gold extraction will occur.

The amount of gold mined depends on the size of the company (DaSilva, 2013). Once raw materials are obtained the next step is gold refining in order to increase the purity of the gold (DaSilva, 2013). The following stages are jewelry design and fabrication and distribution of fabricated jewelry to sellers. The final stage of the input-output structure of the gold industry is gold recycling. The recycling of finished products containing gold makes up roughly one third of the annual supply of gold (DaSilva, 2013). 50% of gold produced annually is used to make jewelry, 40% is held up in investments and the final 10% are used by various industries such as electronics (DaSilva, 2013).

3.2 Market Trends in the Gold Jewelry Industry

The primary gold producers of the world are: South Africa, the United States, Russia, China, Australia, and Peru (DaSilva, 2013). The top 5 exporters of gold jewelry in 2010 were India, China, Italy, USA, and Switzerland. The top 5 importers of gold jewelry in 2010 were China, the United Kingdom, USA, the United Arab Emirates, and Switzerland (DaSilva, 2013). The 2 notable trends in gold jewelry consumption are that: i) the largest consumer markets for gold jewelry are China, India, and the USA and ii) the growth in consumption is occurring in the east for the most part, specifically India and China (DaSilva, 2013).

3.3 Costs of Gold Mining

The World Gold Council (WGC) has established a new cost disclosure framework to prevent mining companies from misleading investors about the cost of business (Whelan, 2013). Previously companies would only report cash costs, the cost of mining and processing. However, these costs do not reflect the true cost incurred from the start to the end of gold mining (Whelan, 2013). The metrics introduced by the WGC include all-in sustaining costs and all-in costs. All-in sustaining costs are an extension of cash costs, however this also includes the costs related to sustaining production (Whelan, 2013). All-in costs include additional costs of mining that vary over the lifecycle of a mine (Whelan, 2013).

3.4 Market Share of the Mining Industry

Due to high entry costs the mining industry roughly 21% of the mining industry is owned by 4 of the largest companies (Adeyemi, 2016). However larger firms require a larger scale of production to remain in business and the risk of large-scale gold mining exceeds that of small-scale gold mining. Due to this difference in risk roughly 25% of gold production originates from artisanal or small-scale mining (DaSilva, 2013).

4| Biosensor Design

4.1 Current Issues in Gold Exploration

As the most accessible sources of gold are being discovered, gold exploration is becoming increasingly difficult despite improvements in geophysical and geochemical techniques (Zammit *et al,* 2013). As a result exploration is now performed in regions where gold is buried deeply underneath the surface. Additionally, in these areas there are haloes of gold and several pathfinder elements found in the overlying soil necessitating the development of new techniques to perform gold exploration in this novel terrain (Zammit *et al*, 2013). Presently the geophysical methods used in gold prospecting are very time consuming. It involves many steps, complex capital, and laboratory analysis. As a result an on-site test to reliably detect the presence of gold would greatly ease the process of gold exploration (Zammit *et al,* 2013).

4.2 Gold Biosensor Introduction

A biosensor is a biological entity that produces a measurable signal upon stimulation by an external stimulus. This signal then provides qualitative and/or quantitative information about the initial stimuli (Zammit *et al*, 2013). Development of biosensing technologies in mining could ease the process of gold exploration by providing a rapid, portable and reliable assay (Zammit *et al*, 2013). Unfortunately, there has been little research into biosensor use in the mining industry (Zammit *et al*, 2013). Development of a gold biosensor requires a biological element that would be responsive to gold (Zammit *et al*, 2013).

4.3 Challenges Implementing a Gold Biosensor

There are several challenges in the development of a commercially applicable gold biosensor. One challenge is that the toxicity of gold to the biological system depends on the concentration and the speciation of the gold in the sample (Zammit *et al*, 2013). Gold doesn't form free ions in aqueous solutions rather it forms aurous (I) or auric (III) complexes. Thus both the speciation and concentration of gold in the sample determine the toxicity and the response of biosensors to the stimulus (Zammit *et al*, 2013). Another challenge is that many microbes have non-specific responses to toxic metals (Zammit *et al*, 2013). The microbial heavy metal defense mechanisms that biosensors would be dependent on to detect gold are often non-specific. As a result these systems will also respond to the presence of other metals and can not be used as a tool for gold exploration (Zammit *et al*, 2013). Furthermore implementation of biosensors in the field is very challenging due to the complex composition of environmental samples (Zammit *et al*, 2013).

4.4 Regulon

The *golTSB* regulon originates from *Salmonella enterica* strain LT2 and it has the potential to be used as the biological basis of a gold biosensor (Zammit *et al*, 2013). GolT is a transmembrane efflux ATPase while GolB acts as a metallochaperone binds to gold complexes to target them for efflux (Zammit *et al*, 2013). GolS controls the transcription of GolT and GolB. Additionally, it has been shown that the *golTSB* regulon has some specificity for Au complexes (Zammit *et al*, 2013). Furthermore, by engineering a construct where the *golTSB* regulon is placed upstream of a promoter-less *lac-Z* reporter gene one can obtain quantitative information about the amount of gold present in a sample (Zammit *et al*, 2013). This construct demonstrated a difference in ability to detect gold based on its speciation (Zammit *et al*, 2013). The *golTSB* - *lac-Z* construct is more sensitive to Au (I) than to Au (III) (Zammit *et al*, 2013). However there was also some cross-reactivity observed with other metals such as Cu (II), Fe (III), Ni (II), Pb (II), and Zn (II) (Zammit *et al*, 2013).

5| Malaria

The fight against malaria is a continuous one where malaria remains endemic in many countries. Malaria is a mosquito-borne disease caused by a parasite. There are five kinds of malaria parasites that infect humans: *Plasmodium falciparum, P. vivax, P. ovale*, *P. knowlesi* and *P. malariae.* Of these, *P. falciparum* is the most virulent and is the predominant strain in Africa. A few common symptoms of malaria are high fevers, shaking chills and flu-like illness (WHO, 2015).

5.1 Miners

Mining has an important role in infrastructure allowing economies to expand and create new riches but with it comes an unfortunate toll. Studies show that areas where mining is prevalent there has been an increase in malaria transmission. These countries include, Ghana, South Africa, Papua New Guinea, Brasil, Colombia, Venezuela, Suriname, and Peru (Castellanos *et al*., 2016).

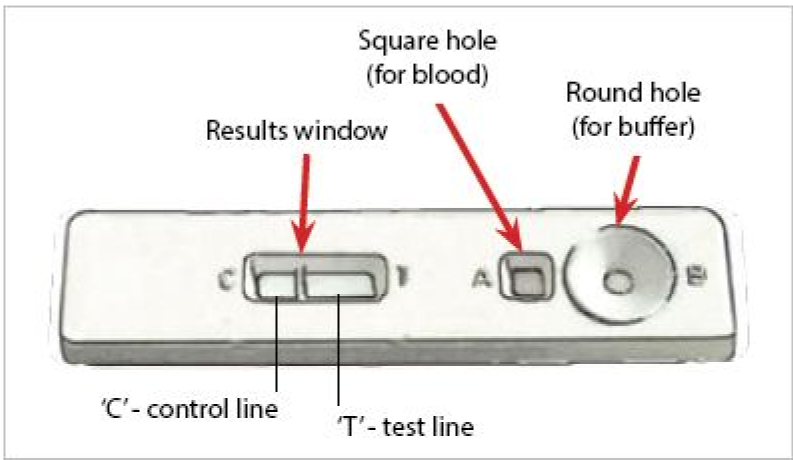
One sector of mining is artisanal small-scale gold mining (ASGM) which is increasing in popularity as there is a higher demand and prices for minerals. ASGM practices cause a subsequent increase in mosquito-borne illnesses amongst mining communities. ASGM employes low-tech methods such as river and land dredging (Lowe, 2006). River dredging techniques apply the force of suction to transport gravel to the surface of the river bed for processing (Dalgety, 2010). Land dredging involves the use of engines and high pressure water jets to suck up loose material into “Sluice Boxes” where gold is trapped (Dalgety, 2010). No-tech methods using a shovel and a pan are also still in practice. These mining methods result in open pits that are often neglected and filled with rainwater (International Human Rights Clinic, 2007). These sites are preferred breeding ground for mosquitoes, which puts miners at a high risk for contracting malaria, and later spreading it to their own community, as well as other communities (CASM, 2003).

5.2 Rapid Diagnostic Tests (RDTs)

Malaria diagnosis has been one of many effective ways to prevent the spread of malaria and allows communities to plan and strategize for next steps in dealing with malaria. In order for diagnosis’ to be effective individuals who can carry malaria must be recognized promptly in order to prevent spread of infection. There are several ways to diagnose a patient. Clinical diagnoses are based on initial symptoms. Microscopic diagnosis using examination of blood smear which is strained. Most test are in dipstick or cassette format which has results ready within fifteen minutes (Wilson 2012).

In the market today, there are over 200 malaria RDT products and the use of such products have been consistently increasing in recent years (Wilson 2012). In 2010 alone there were approximately 50 million malaria RDTs distributed. Some can detect only single species and other can distinguish between species or detect multiple species (Wilson 2012). WHO ensures RDTs consistently detect over 75 percent of low parasite density samples, have false positive rate of less than ten percent and have invalid tests of less than five percent (Rapid Diagnostic Tests, 2016).

RDTs detect specific proteins produced by malaria parasites in the blood of infected individuals (Wilson 2012). Blood for the test is retrieved by pricking the finger.



The first step of the test is to mix the patient's blood with a lysing agent in a test strip or well. This breaks the membrane surrounding the red blood cells and releases more parasite protein (Wilson 2012). Afterwards a antibody that is dyed targets a specific antigen and is bound to the test line. Blood and buffer is then mixed with the antibody. If an antigen is present it's trapped on the test line (Wilson 2012). The intensity of the bands will vary with the amount of antigen present. Current malaria RDTs are based on the detection of 3 different types of *Plasmodium* antigens: i) *Plasmodium* histidine rich protein 2 (pHRP-2) which can be specific for *P. falciparum* or *P. vivax* ii) *Plasmodium* lactate dehydrogenase (pLDH) which is specific for *P. falciparum, P. vivax,* or can be panspecific (a variant common to all strains of *Plasmodium*) iii) *Plasmodium* aldolase which is panspecific (Wilson 2012). The combination of these 3 antigens allows for detection of any species of *Plasmodium*.

Since malaria RDTs are dependent on these 3 antigens they have several diagnostic limitations. None of the antigens are specific to *P. knowlesi, P. ovale,* or *P. malariae*. There are *P. falciparum* strains in South America that use uncommon pHRP variants meaning RDTs based

on detection of pHRP-2 would not be useful in these regions (Wilson 2012). Occasionally cross-reactions are observed between *Schistosoma mekongi* infections and pHRP-2 assays (Wilson 2012). Cross-reactions have also been observed for patients with various circulating auto-antibodies (Wilson 2012). Patients with high levels of *P. falciparum* may generate false positives in pLDH assays testing for the presence of *P. vivax* (Wilson 2012). Malaria RDTs can not be used to quantify the magnitude of parasitemia (Wilson 2012). Lastly, malaria RDTs can not be used to measure response to therapy because it takes roughly 30 days for pHRP-2 to be cleared from blood (Wilson 2012).

RDTs also exist for other infectious diseases such as syphilis and HIV, both of which are major killers in underdeveloped nations. Rural populations in these countries don't have access to diagnostic labs so infected individuals are typically not diagnosed until symptom onset occurs (Mabey *et al.* 2012). This is an issue for infections with a latency phase such as syphilis and HIV as earlier detection can make a tremendous difference in the success of treatment. RDTs are available that don't require a lab, electricity or skilled personnel and thus are a potential solution to this issue (Mabey *et al.* 2012).

However, there are barriers to the implementation of these RDTs that must be addressed. Health care workers (HCWs) must be trained to correctly use and interpret these tests, the quality of testing has to be maintained, supply chains must be improved so that RDTs are continuously available (Mabey *et al.* 2012). It was found that periodic retraining of HCWs resulted in maintenance of high quality testing over an 18 month period (Mabey *et al.* 2012). Furthermore, these syphilis RDTs were accepted and deemed easy to use by HCWs in the countries they were introduced into (Mabey *et al.* 2012). Implementation of syphilis RDTs also increased the proportion of women attending an antenatal clinic that are screened for syphilis from 40% to over 90% (Mabey *et al.* 2012). This success has led to change in the national policies of the 6 nations. Following this study, all 6 of these countries changed their policy to recommend use of RDTs. Additionally this research has led some neighboring countries to implement RDTs (Mabey *et al.* 2012). Thus, although there are still many unanswered questions about RDTs they are essential in the diagnosis and early detection of infectious disease in impoverished areas.

6| Implementation Framework

When forming our preliminary design, we aimed at creating individual kits that would consist of the gold biosensor mounted at the top. Artisanal miners would apply their soil sample and be able to view the concentration of gold ions present in real time using the smartphone app.Inside the kit are supplies needed to conduct an RDT such as a test packet, lancet, alcohol swab, cotton ball, buffer and gloves. To conduct the test, miners would prick their 4th finger using the blood-transfer device in the test packet, and place it on the test cassette at the top. Miners would then place buffer in the test cassette and wait to receive test results.

The issue we came across in this design process is preparing the soil sample for the paper biosensor. Since the gold biosensor works by detecting the presence of Au ions, the soil sample would need to be centrifuged to remove large debris and treated with aqua regia (a mixture of HCL and nitric acid). We then tried to move the design to a lab-on-wheels model where our criteria would still be valid, however we realized that transporting aqua regia is not feasible and would produce undesired outcomes if in the wrong hands.

Our team discussed the possibility of an even broader design that would no longer be physical- but rather rely on the artisanal mining sector collaborate with junior mining companies who would have the resources to purchase and distribute the biosensor and offer community health clinics. Our implementation framework is based on an incentive model whereby both parties are motivated through self-interest. The process is as follows:

Step 1: Junior mining companies (who have the financial resources) buy the biosensor and are in control of production/distribution.

Step 2: Junior mining companies collaborate with Artisanal and Small-Scale Gold miners (ASGM)

Step 3: ASGMs are given access to the gold biosensor

Step 4: Junior mining companies require that ASGMs take a malaria RDT prior to receiving the soil sample results on the paper-based biosensor

Junior Mining Companies would be motivated to participate in this incentive model because ASGM makes up 20-30% of the mining industry and can allow them increased revenue. Artisanal and small-scale gold miners would also be likely to cooperate in this model due to potential for secure employment, increased rights to mining land, union opportunities/resources that are available to Junior Mining Companies.

Using the malaria RDT could benefit Junior Mining Companies due to ensuring that artisanal miners are healthy and can work more efficiently. If the test is positive, arrangements can be made through internal health clinics who can treat artisanal miners more effectively and minimize misdiagnosis and therefore improper malaria treatment. From the point-of-view from ASGM, being provided with a malaria RDT could allow access to useful information about their own health status and the health risks of their families.

Potential problems that would equally be likely is the possibility of Junior Mining Companies exploiting ASGMs (since they make up a substantial amount of the industry) by taking ownership of artisanal miners who would have otherwise had the complete share of the gold they mine.

There is also potential for unethical employment equity where Junior Mining Companies only reveal sample results to ASGMs who have tested negative for malaria. Conversely, ASGMs could serve as a burden to Junior Mining Companies if tested positive because Junior Mining Companies are now responsible for treatment as well as finding other miners to make up for time away from work.

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